Amendments to the Claims

This listing of claims will replace all prior versions, and listings, of claims in the application:

1. (currently amended) A fibronectin type III (Fn3) polypeptide monobody comprising:

at least two Fn3 β-strand domain sequences with a loop region sequence linked between adjacent β-strand domain sequences; and

optionally, an N-terminal tail of at least about 2 amino acids, a C-terminal tail of at least about 2 amino acids, or both;

wherein at least one loop region sequence, the N-terminal tail, or the C-terminal tail comprises an amino acid sequence which varies by deletion, insertion, or replacement of at least two amino acids from a corresponding loop region, N-terminal tail, or C-terminal tail in a tenth ~~wild-type~~ Fn3 domain of fibronectin, and

wherein the polypeptide monobody exhibits nuclear receptor binding activity.

2. (original) The polypeptide monobody according to claim 1, wherein the nuclear receptor is selected from the group consisting of steroid receptors, thyroid receptors, retinoid receptors, vitamin D receptors, and orphan nuclear receptors.

3. (original) The polypeptide monobody according to claim 2, wherein the nuclear receptor is a steroid receptor.

4. (previously presented) The polypeptide monobody according to claim 3, wherein the steroid receptor is an estrogen receptor, an androgen receptor, a progestin receptor, a glucocorticoid receptor, or a mineralocorticoid receptor.

5. (original) The polypeptide monobody according to claim 4, wherein the steroid receptor is an estrogen receptor.

6. (original) The polypeptide monobody according to claim 5, wherein the polypeptide monobody exhibits estrogen receptor binding activity in the presence of an estrogen receptor agonist or an estrogen receptor antagonist.

7. (original) The polypeptide monobody according to claim 6, wherein the estrogen receptor agonist is estradiol, estriol, diethylstilbestrol, or genistein.

8. (original) The polypeptide monobody according to claim 6, wherein the estrogen receptor antagonist is hydroxy tamoxifen, ICI182780, or raloxifene.

9. (currently amended) The polypeptide monobody according to claim 1, wherein said at least two Fn3 β-strand domain sequences comprises β-strand domain sequences A through G of a ~~wild-type~~ tenth Fn3 domain of human fibronectin or derivatives thereof, wherein the loop region sequences comprise an AB loop, a BC loop, a CD loop, a DE loop, an EF loop, and an FG loop.

10. (previously presented) The polypeptide monobody according to claim 9, wherein the at least one loop region sequence is selected from the group consisting of the AB loop region sequence, the BC loop region sequence, the DE loop region sequence, the FG loop region sequence, and combinations thereof.

11. (original) The polypeptide monobody according to claim 9, wherein the at least one loop region sequence is a combination of the BC loop region sequence and the FG loop region sequence.

12. (currently amended) The polypeptide monobody according to claim 1, wherein the tenth ~~wild-type~~ Fn3 domain of fibronectin is a ~~wild-type~~ tenth Fn3 domain of human fibronectin.

13. (original) A fusion protein comprising:

a first portion comprising a polypeptide monobody according to claim 1 and

a second portion fused to the first portion.

14. (original) The fusion protein according to claim 13, wherein the second portion comprises a label.

15. (original) The fusion protein according to claim 14, wherein the label is an alkaline phosphatase tag or a $His_{(6)}$ tag.

16. (original) The fusion protein according to claim 13, wherein the second portion comprises a transcriptional activation domain.

17–108. (cancelled)

109. (withdrawn) An *in vivo* composition comprising:

a fusion polypeptide according to claim 16; and

a second fusion polypeptide comprising a target protein, or fragment thereof, fused to the C-terminus of a DNA-binding domain that binds to a 5' regulatory region of a reporter gene, wherein said target protein or fragment thereof comprises a nuclear receptor, or fragment thereof, said nuclear receptor or fragment thereof including a ligand-binding domain;

wherein binding of the polypeptide monobody of the fusion polypeptide to the target protein, or fragment thereof, of the second fusion polypeptide brings the transcriptional activation domain of the fusion polypeptide in sufficient proximity to the DNA-binding domain of the second fusion polypeptide to induce expression of the reporter gene.

110. (withdrawn) The *in vivo* composition according to claim 109, wherein the nuclear receptor is selected from the group consisting of steroid receptors, thyroid receptors, retinoid receptors, vitamin D receptors, and orphan nuclear receptors.

111. (withdrawn) The *in vivo* composition according to claim 110, wherein the nuclear receptor is a steroid receptor.

112. (withdrawn) The *in vivo* composition according to claim 111, wherein the steroid receptor is an estrogen receptor, an androgen receptor, a progesterone receptor, a glucocorticoid receptor, or a mineralocorticoid receptor.

113. (withdrawn) The *in vivo* composition according to claim 112, wherein the steroid receptor is an estrogen receptor.

114. (withdrawn) The *in vivo* composition according to claim 109, wherein the reporter gene is a nutrient marker gene, a β-galactosidase gene, or a fluorescent protein gene.

115. (withdrawn-currently amended) The *in vivo* composition according to claim 109, wherein the at least two Fn3 β-strand domain sequences comprises β-strand domain sequences A through G of a ~~wild-type~~ tenth Fn3 domain of human fibronectin or derivatives thereof, wherein the loop region sequences comprise an AB loop, a BC loop, a CD loop, a DE loop, an EF loop, and an FG loop.

116. (withdrawn) The *in vivo* composition according to claim 115, wherein the at least one loop region sequence is selected from the group consisting of the AB loop region sequence, the BC loop region sequence, the DE loop region sequence, the FG loop region sequence, and combinations thereof.

117. (withdrawn) The *in vivo* composition according to claim 109, wherein the amino acid sequence is at least about 5 amino acids in length.

118. (withdrawn) The *in vivo* composition according to claim 109, wherein the amino acid sequence is at least about 10 amino acids in length.

119. (withdrawn) The *in vivo* composition according to claim 109, wherein the *in vivo* composition is present in a bacteria, a mammalian cell, or a yeast cell.

120. (withdrawn) The *in vivo* composition according to claim 109, wherein the transcriptional activation domain is a B42 activation domain or a Gal4 activation domain.

121. (withdrawn) The *in vivo* composition according to claim 109, wherein the DNA-binding domain is a LexA DNA-binding domain or a Gal4 DNA-binding domain.

122. (withdrawn) A method of screening a candidate drug for nuclear receptor agonist or antagonist activity, said method comprising:

providing a host cell comprising (i) a reporter gene under control of a 5' regulatory region, (ii) a first fusion polypeptide comprising a nuclear receptor, or fragment thereof including a ligand-binding domain, fused to a C-terminus of a DNA-binding domain that binds to the 5' regulatory region of the reporter gene, and (iii) a second fusion polypeptide according to claim 16, wherein said polypeptide monobody includes a polypeptide sequence that binds to the nuclear receptor, or fragment thereof, in the absence of both an agonist and an antagonist of the nuclear receptor, presence of an agonist of the nuclear receptor, presence of an antagonist of the nuclear receptor, or presence of both an agonist and an antagonist of the nuclear receptor;

growing the host cell in a growth medium comprising a candidate drug; and

detecting expression of the reporter gene, which indicates binding of the polypeptide sequence of the second fusion polypeptide to the nuclear receptor, or fragment thereof, such that the transcriptional activation domain of the second fusion polypeptide is in sufficient proximity to the DNA-binding domain of the first fusion polypeptide to allow expression of the reporter gene,

wherein modulation of reporter gene expression indicates that the candidate drug is either an agonist or an antagonist, or has mixed activity.

123. (withdrawn) The method according to claim 122, wherein an increase in reporter gene expression indicates that the candidate drug has agonist activity when the polypeptide sequence binds to the nuclear receptor, or fragment thereof, in the presence of an agonist.

124. (withdrawn) The method according to claim 122, wherein an increase in reporter gene expression indicates that the candidate drug has antagonist activity when the polypeptide sequence binds to the nuclear receptor, or fragment thereof, in the presence of the antagonist.

125. (withdrawn) The method according to claim 122, wherein said providing comprises providing four host cells and separately growing the four host cells on the same growth media containing the same candidate drug, and

wherein the first host cell comprises a second fusion polypeptide including a polypeptide sequence that binds the nuclear receptor in the presence of only a nuclear receptor agonist,

wherein the second host cell comprises a second fusion polypeptide including a polypeptide sequence that binds the nuclear receptor in the presence of only a nuclear receptor antagonist,

wherein the third host cell comprises a second fusion polypeptide including a polypeptide sequence that binds the nuclear receptor in the presence of both a nuclear receptor agonist and a nuclear receptor antagonist, and

wherein the fourth host cell comprises a second fusion polypeptide including a polypeptide sequence that binds the nuclear receptor in the presence of neither a nuclear receptor agonist nor a nuclear receptor antagonist.

126. (withdrawn) The method according to claim 122, wherein the polypeptide monobody is derived from a tenth fibronectin type III domain of human fibronectin.

127. (withdrawn) The method according to claim 122, wherein the polypeptide sequence is present in a loop region sequence, N-terminal tail, or C-terminal tail of the polypeptide monobody.

128. (withdrawn) The method according to claim 122, wherein the transcriptional activation domain is a B42 or a Gal4 activation domain.

129. (withdrawn) The method according to claim 122, wherein the DNA-binding domain is a LexA DNA-binding domain or a Gal4 DNA-binding domain.

130. (withdrawn) The method according to claim 122, wherein the reporter gene is a nutrient marker gene, a β-galactosidase gene, or a fluorescent protein gene.

131. (withdrawn) The method according to claim 130, wherein the reporter gene is a nutrient marker gene and said detecting comprises exposing host cells to a nutrient-deficient media and identifying host cell colonies that grow on the nutrient-deficient media.

132. (withdrawn) The method according to claim 130, wherein the reporter gene is a β-galactosidase gene and said detecting comprises exposing host cells to X-gal and identifying host cell colonies exhibiting β-galactosidase activity.

133. (withdrawn) The method according to claim 130, wherein the reporter gene is a fluorescent protein gene and said detecting comprises exposing the host cells to an excitatory light source and identifying host cells that emit light at a particular wavelength.

134. (withdrawn) The method according to claim 122, wherein the nuclear receptor is selected from the group consisting of steroid receptors, thyroid receptors, retinoid receptors, vitamin D receptors, and orphan nuclear receptors.

135. (withdrawn) The method according to claim 134, wherein the nuclear receptor is a steroid receptor.

136. (withdrawn) The method according to claim 135, wherein the steroid receptor is an estrogen receptor, an androgen receptor, a progesterone receptor, a glucocorticoid receptor, or a mineralocorticoid receptor.

137. (withdrawn) The method according to claim 122, wherein the host cell is a bacteria, a mammalian cell, or a yeast cell.

138. (withdrawn) A kit comprising:

a culture system which includes a culture medium on which has been placed at least one transformed host cell, each of the at least one transformed host cell comprising (i) a reporter gene under control of a 5' regulatory region, (ii) a first fusion polypeptide comprising a nuclear receptor, or fragment thereof, including a ligand-binding domain, fused to a C-terminus of a DNA-binding domain that binds to the 5' regulatory region of the reporter gene, and (iii) a second fusion polypeptide according to claim 16,

wherein said polypeptide monobody includes a polypeptide sequence that binds to the nuclear receptor, or fragment thereof, in the absence of both an agonist and an antagonist of the nuclear receptor, presence of an agonist of the nuclear receptor, presence of an antagonist of the nuclear receptor, or presence of both an agonist and an antagonist of the nuclear receptor.

139. (withdrawn) The kit according to claim 138, wherein the at least one type of transformed host cell comprises:

a first transformed host cell comprising a second fusion polypeptide where the polypeptide sequence binds to the nuclear receptor, or fragment thereof, in the presence of a nuclear receptor agonist, and

a second transformed host cell comprising a second fusion polypeptide where the polypeptide sequence binds to the nuclear receptor, or fragment thereof, in the presence of a nuclear receptor antagonist.

140. (withdrawn) The kit according to claim 139, wherein the first and second transformed host cells are strategically placed on the growth medium such that the first and second transformed host cells are physically separated from one another.

141. (withdrawn) The kit according to claim 139, wherein the at least one transformed host cell further comprises:

a third transformed host cell comprising a second fusion polypeptide where the polypeptide sequence binds to the nuclear receptor, or fragment thereof, in the absence of both an agonist and an antagonist; and

a fourth transformed host cell comprising a second fusion polypeptide where the polypeptide sequence binds to the nuclear receptor, or fragment thereof, in the presence of both an agonist and an antagonist.

142. (withdrawn) The kit according to claim 141, wherein the third and fourth transformed host cells are strategically placed on the growth medium such that the third and fourth transformed host cells are physically separated from one another and from the first and second transformed host cells.

143. (withdrawn) The kit according to claim 138, wherein the polypeptide monobody is derived from a tenth fibronectin type III domain of human fibronectin.

144. (withdrawn) The kit according to claim 138, wherein the host cell is a bacteria, a mammalian cell, or a yeast cell.

145. (withdrawn) A method of validating nuclear receptor protein activity comprising:

exposing a nuclear receptor protein to a polypeptide monobody according to claim 1 which binds to the nuclear receptor protein and

determining whether binding of the nuclear receptor protein by the polypeptide monobody modifies nuclear receptor protein activity.

146. (withdrawn) The method according to claim 145, wherein said exposing is carried out *in vivo*.

147. (withdrawn) The method according to claim 146, wherein said exposing is carried out in a yeast cell, bacterial cell, or mammalian cell.

148. (withdrawn) The method according to claim 145, wherein said determining comprises:

detecting mRNA or protein expression levels prior to said exposing and after said exposing and

comparing the detected mRNA or protein expression levels to identify proteins that are downstream of the pathway of the nuclear receptor protein, wherein modified expression levels indicated modified nuclear receptor protein activity.

149. (withdrawn) The method according to claim 145, wherein the nuclear receptor protein is required for cell growth or survival, said determining comprising:

measuring cell growth or survival after said exposing, wherein reduced cell growth or survival indicates inhibition of nuclear receptor protein activity.

150. (withdrawn) The method according to claim 145, wherein the nuclear receptor protein is a pathogen protein involved in host-pathogen interaction, said exposing comprising:

exposing a host cell comprising the polypeptide monobody to the pathogen.

151. (withdrawn) The method according to claim 150, wherein said determining comprises:

determining the extent of pathogen-induced disease progression in the host cell.

152. (withdrawn) The method according to claim 150, wherein the pathogen is a bacteria.

153. (withdrawn) A method of measuring polypeptide monobody binding affinity for a nuclear receptor protein, said method comprising:

exposing a nuclear receptor protein to (i) an interaction partner that binds the nuclear receptor protein, and (ii) a polypeptide monobody according to claim 1 that binds the nuclear receptor protein; and

measuring the degree to which the polypeptide monobody competes with the interaction partner.

154. (withdrawn) The method according to claim 153, wherein said exposing is carried out *in vitro*.

155. (withdrawn) The method according to claim 154, wherein the nuclear receptor protein is bound to a substrate.

156. (withdrawn) The method according to claim 154, wherein the polypeptide monobody comprises a label.

157. (withdrawn) The method according to claim 156, wherein the label is an alkaline phosphatase tag or a $His_{(6)}$ tag.

158. (withdrawn) The method according to claim 153, wherein said exposing is carried out *in vivo*.

159. (withdrawn) A method of modulating nuclear receptor protein activity comprising:

exposing a nuclear receptor protein to a polypeptide monobody according to claim 1 that binds the nuclear receptor protein under conditions effective to modify nuclear receptor protein activity.

160. (withdrawn) The method according to claim 159, wherein said exposing is carried out *in vivo*.

161. (withdrawn) The method according to claim 160, wherein said exposing is carried out in a yeast cell, bacterial cell, or mammalian cell.

162. (withdrawn) The method according to claim 159, wherein the nuclear receptor is selected from the group consisting of steroid receptors, thyroid receptors, retinoid receptors, vitamin D receptors, and orphan nuclear receptors.

163. (withdrawn) The method according to claim 162, wherein the nuclear receptor is a steroid receptor.

164. (withdrawn) The method according to claim 163, wherein the steroid receptor is an estrogen receptor, an androgen receptor, a progestin receptor, a glucocorticoid receptor, or a mineralocorticoid receptor.

165. (withdrawn) The method according to claim 164, wherein the steroid receptor is an estrogen receptor.

166. (withdrawn) A method of detecting conformation of a nuclear receptor protein, said method comprising:

exposing a nuclear receptor protein to a polypeptide monobody according to claim 1 that interacts with the nuclear receptor protein when the nuclear receptor protein is in a specific conformation, under conditions effective for the polypeptide monobody to interact with the nuclear receptor protein, and

determining whether the polypeptide monobody interacts with the nuclear receptor protein, wherein interaction between the polypeptide monobody and the nuclear receptor protein indicates that the nuclear receptor protein is in the specific conformation.

167. (withdrawn) The method according to claim 166, wherein said exposing is carried out *in vitro*.

168. (withdrawn) The method according to claim 167, wherein the nuclear receptor protein is bound to a substrate.

169. (withdrawn) The method according to claim 167, wherein the polypeptide monobody comprises a label.

170. (withdrawn) The method according to claim 169, wherein the label is an alkaline phosphatase tag or a $His_{(6)}$ tag.

171. (withdrawn) The method according to claim 166, wherein said exposing is carried out *in vivo*.

172. (withdrawn) The method according to claim 171, wherein said exposing is carried out in a yeast cell, bacterial cell, or mammalian cell.

173. (withdrawn) The method according to claim 166, wherein the nuclear receptor is selected from the group consisting of steroid receptors, thyroid receptors, retinoid receptors, vitamin D receptors, and orphan nuclear receptors.

174. (withdrawn) The method according to claim 173, wherein the nuclear receptor is a steroid receptor.

175. (withdrawn) The method according to claim 174, wherein the steroid receptor is an estrogen receptor, an androgen receptor, a progestin receptor, a glucocorticoid receptor, or a mineralocorticoid receptor.

176. (withdrawn) The method according to claim 175, wherein the steroid receptor is an estrogen receptor.

177. (withdrawn) A method of detecting a change in conformation of a nuclear receptor protein, said method comprising:

(i) detecting conformation of a nuclear receptor protein according to the method of claim 166; and

(ii) repeating said detecting after a time delay to determine whether the nuclear receptor protein binds to a different polypeptide monobody or to the same polypeptide monobody but with a different degree of interaction;

wherein binding to a different polypeptide monobody or a change in degree of interaction with the same polypeptide monobody indicates change in conformation of the nuclear receptor protein.

178. (withdrawn) The method according to claim 177, further comprising exposing the nuclear receptor protein to a ligand prior to step (ii).

179. (withdrawn) The method according to claim 178, wherein said exposing is carried out *in vitro*.

180. (new) The polypeptide monobody according to claim 1, wherein the FG loop region sequence comprises the amino acid sequence selected from the group of SEQ ID NO: 20 and SEQ ID NO: 32.

181. (new) The polypeptide monobody according to claim 1, wherein the BC loop region sequence comprises the amino acid sequence selected from the group of SEQ ID NO: 22, SEQ ID NO: 23, and SEQ ID NO: 24.

182. (new) The polypeptide monobody according to claim 1, wherein the FG loop region sequence comprises the amino acid sequence selected from the group of SEQ ID NO: 25, SEQ ID NO: 26, SEQ ID NO: 27, SEQ ID NO: 28, SEQ ID NO: 29, SEQ ID NO: 30, SEQ ID NO: 31, SEQ ID NO: 36, SEQ ID NO: 37, SEQ ID NO: 38, SEQ ID NO: 39, SEQ ID NO: 40, SEQ ID NO: 41, SEQ ID NO: 42, SEQ ID NO: 43, SEQ ID NO: 44, SEQ ID NO: 45, SEQ ID NO: 46, SEQ ID NO: 47, SEQ ID NO: 48, SEQ ID NO: 49, SEQ ID NO: 50, SEQ ID NO: 51, SEQ ID NO: 52, SEQ ID NO: 53, SEQ ID NO: 54, SEQ ID NO: 55, SEQ ID NO: 56, SEQ ID NO: 57, SEQ ID NO: 58, SEQ ID NO: 59, SEQ ID NO: 60, SEQ ID NO: 61, SEQ ID NO: 62, SEQ ID NO: 63, SEQ ID NO: 64, SEQ ID NO: 65, SEQ ID NO: 66, SEQ ID NO: 67, SEQ ID NO: 68, SEQ ID NO: 69, SEQ ID NO: 70, SEQ ID NO: 71, SEQ ID NO: 72, and SEQ ID NO: 73.

183. (new) The polypeptide monobody according to claim 1, wherein the AB loop region sequence comprises the amino acid sequence selected from the group of SEQ ID NO: 34 and SEQ ID NO: 35.

184. (new) The polypeptide monobody according to claim 1, wherein the Fn3 domain of fibronectin has an amino acid sequence of SEQ ID NO: 2 or SEQ ID NO: 3.